Chromatin-Bound Cullin-Ring Ligases: Regulatory Roles in DNA Replication and Potential Targeting for Cancer Therapy

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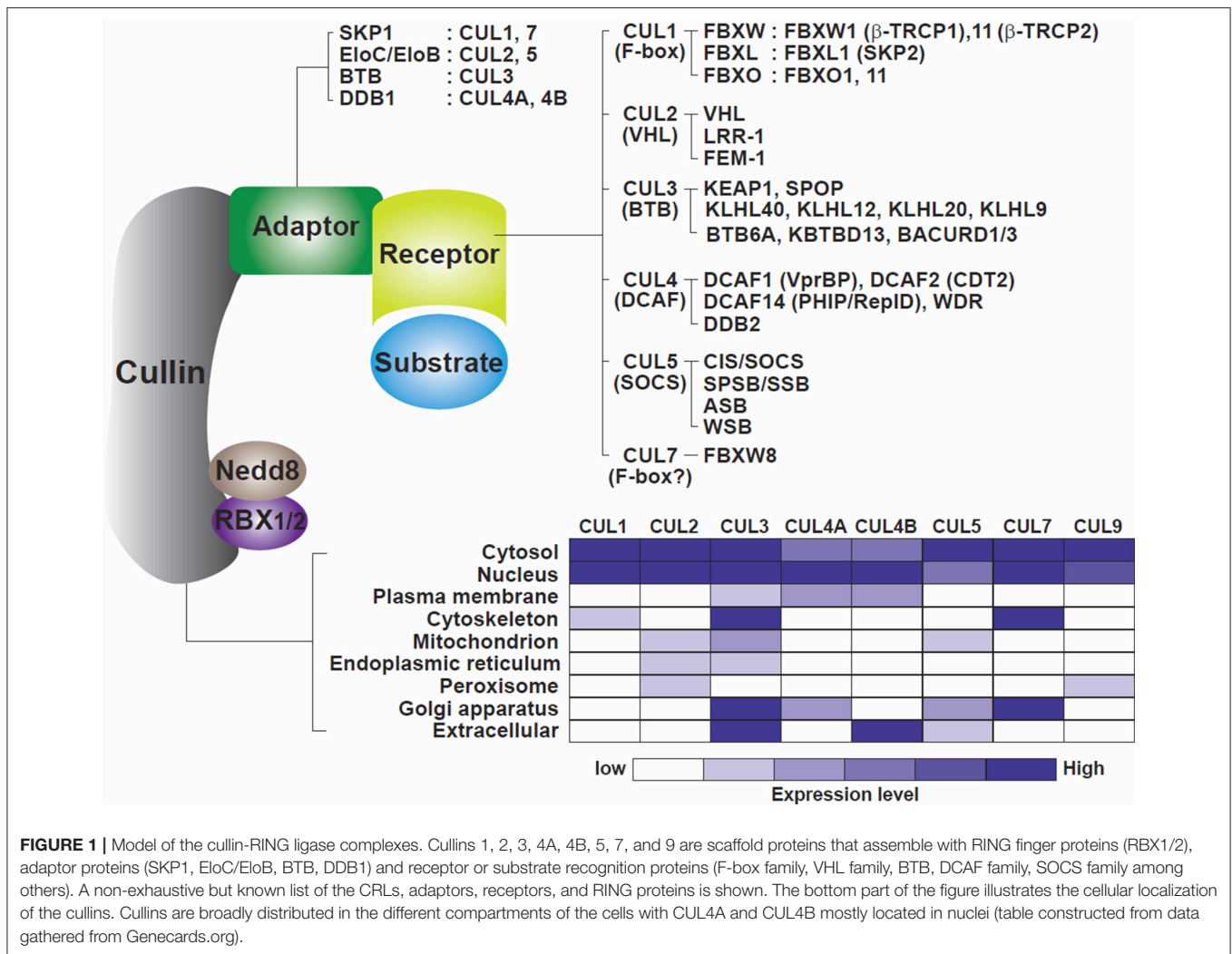
Cullin-RING (Really Interesting New Gene) E3 ubiquitin ligases (CRLs), the largest family of E3 ubiquitin ligases, are functional multi-subunit complexes including substrate receptors, adaptors, cullin scaffolds, and RING-box proteins. CRLs are responsible for ubiquitination of ∼20% of cellular proteins and are involved in diverse biological processes including cell cycle progression, genome stability, and oncogenesis. Not surprisingly, cullins are deregulated in many diseases and instances of cancer. Recent studies have highlighted the importance of CRL-mediated ubiquitination in the regulation of DNA replication/repair, including specific roles in chromatin assembly and disassembly of the replication machinery. The development of novel therapeutics targeting the CRLs that regulate the replication machinery and chromatin in cancer is now an attractive therapeutic strategy. In this review, we summarize the structure and assembly of CRLs and outline their cellular functions and their diverse roles in cancer, emphasizing the regulatory functions of nuclear CRLs in modulating the DNA replication machinery. Finally, we discuss the current strategies for targeting CRLs against cancer in the clinic.

Keywords: DNA replication, chromatin, ubiquitin ligases, cancer, therapy

STRUCTURE AND REGULATION OF CRLs

CRLs are composed of four components (**Figure 1**): cullins as molecular scaffolds, adaptor proteins, substrate receptors at the N-termini of cullins, and RING proteins at the C-termini of cullins, recruiting ubiquitin-loaded E2 enzymes (Bulatov and Ciulli, 2015). The evolutionarily conserved cullin family encompasses eight key members (CUL1, 2, 3, 4A, 4B, 5, 7, and 9) that exhibit similar structural architectures and contain cullin homology domains (Sarikas et al., 2011). Activation of CRLs is commonly regulated by NEDD8 modifications at lysine residues located at the C-termini of cullins (Soucy et al., 2010). Otherwise, individual CRLs include specific components, employing substrate receptors as critical determinants of substrate specificity.

CRL1, also known as SCF (SKP1-Cullin 1-F box protein), utilizes S-phase kinase-associated protein 1 (SKP1) as an adaptor protein and recognizes its substrates through substrate recognition proteins known as F-box proteins, which contain 40-amino-acid F-box domains (Zheng et al., 2002). Sixty nine F-box proteins are known to be encoded by the human genome to date and are classified into sub-groups based on their different substrate binding domains, including FBXW (F-box and WD40 domains) FBXL (F-box and leucine-rich repeats) and FBXO (F-box only) (Skaar et al., 2013). CRL2 and CRL5 share an identical adaptor, Elongin C (EloC), known to enhance

FIGURE 1 | Model of the cullin-RING ligase complexes. Cullins 1, 2, 3, 4A, 4B, 5, 7, and 9 are scaffold proteins that assemble with RING finger proteins (RBX1/2), adaptor proteins (SKP1, EloC/EloB, BTB, DDB1) and receptor or substrate recognition proteins (F-box family, VHL family, BTB, DCAF family, SOCS family among others). A non-exhaustive but known list of the CRLs, adaptors, receptors, and RING proteins is shown. The bottom part of the figure illustrates the cellular localization of the cullins. Cullins are broadly distributed in the different compartments of the cells with CUL4A and CUL4B mostly located in nuclei (table constructed from data gathered from Genecards.org).

the rate of RNA polymerase II elongation (Bradsher et al., 1993), and utilize either von Hippel-Lindau (VHL) or suppressors of cytokine signaling (SOCS)-box proteins as distinct substrate receptors (Muniz et al., 2013; Cardote et al., 2017). CRL3 interacts with several BTB (Bric-a-brac, Tramtrack, Broad-complex) domain-containing proteins that implement dual functions as adaptor and receptor subunits (Pintard et al., 2004). The BTB domains of these subunits act as adaptors by associating with CUL3 and RBX1, whereas their MATH (meprin and TRAF homology) motifs and Kelch beta-propeller repeat and zinc finger motifs recognize the substrates (Stogios et al., 2005). CRL4 is anchored by two highly similar scaffold proteins, CUL4A and CUL4B, and an adapter, DDB1 (damage-specific DNA binding protein 1). DDB1 contains three WD40 propeller domains (BPA, BPB, and BPC) and links the CUL4 scaffold with multiple substrate receptors termed DCAFs (DDB1-CUL4-associated factors). Over 100 DCAFs have been identified to date (Zimmerman et al., 2010; Harper and Tan, 2012). CRL7 and CRL9 contain the two largest cullin scaffold proteins, CUL7 (1698 amino acids) and CUL9 (2517 amino acids). As these two cullins are much larger than the cullins anchoring

the other CRLs (745–913 amino acids), CRL7/9 may have additional specific, unique functions and/or protein partners. CRL7 is similar to CRL1 in that it includes SKP1 as an adaptor and FBXW8 as a substrate receptor (Dias et al., 2002; Sarikas et al., 2008), but unlike CUL1, it does not interact with the adaptor/receptor complexes SKP1/βTRCP2 or SKP1/SKP2 (Dias et al., 2002). These variations delineate distinct ubiquitin-dependent proteolysis pathways that may be involved in the degradation of specific substrates involved in specific cellular processes and /or in specific cell compartments. Cellular activities of the different CRLs can be co-regulated. For example, CUL7 (with OBSL1 and CCDC8) regulates CUL9 and its substrates to maintain genome stability (reviewed in Jackson, 2014) while degradation of the CRL4 component CDT2 can be orchestrated by the CRL1 (CUL1/FBXO11) complex (Abbas et al., 2013; Abbas and Dutta, 2017).

CRLs are involved in diverse biological processes including cell cycle control, DNA replication, DNA-damage repair, and chromatin remodeling through the selective degradation of various protein substrates, mediated by specific interactions with various substrate receptors. As an example, the SCF (CRL1)

complex plays a vital regulatory role by ubiquitinating a series of cell cycle regulators including EMI1 (Margottin-Goguet et al., 2003), CDC25A and B (Busino et al., 2003; Kanemori et al., 2005), WEE1A (Watanabe et al., 2004), Cyclin D1 (Wei et al., 2008), and PTTG1/Securin (Limón-Mortés et al., 2008). These substrates are recognized by F-box proteins that bind consensus sequences such as D-pS/pT-G-X-X-pS and/or **D**/E/S-**S**/E/D-**G**/A-x_{2-4}-**S**/E/D for β-TrCP (Limón-Mortés et al., 2008) (Hansen et al., 2004) or 0X000S/TPXXS/T/E for FBW7 (Limón-Mortés et al., 2008; Wertz et al., 2011) (X, 0 = random or hydrophobic amino acids, respectively). F-box proteins exhibit a high affinity for serine or threonine residues phosphorylated by specific kinases such as JNK, p38, and CKII (Limón-Mortés et al., 2008; Wertz et al., 2011). While the assembly of cullins, adaptors, and substrate receptors into multiple combinations is necessary for specific arrays of cellular biological responses through time and space, such combinatorial complexity is a major challenge for understanding CRLs' roles in cell signaling and diseases. Some substrates of CRL targeted ubiquitination and their roles are listed in **Table 1**.

CRLs associate with the small protein NEDD8 (Neural precursor cell expressed developmentally down-regulated protein 8) and this interaction is essential for their ubiquitin ligase activities. NEDDylation is accomplished by the sequential action of an NAE (Nedd8-activating enzyme) and a Nedd8-conjugating enzyme, UBC12 (Haas, 2007). CRLs can be deNEDDylated by the zinc-dependent metalloenzyme CSN5, a component of the COP9 signalosome (CSN) complex, which cleaves the isopeptidic bond between cullin and NEDD8 (Cope and Deshaies, 2003). NEDDylation is also regulated by CAND1 (Cullin-associated Nedd8-dissociated protein 1), which binds to unneddylated cullins, inhibiting NEDD8 conjugation and consequently resulting in inhibition of both cullin NEDDylation and CRLs activities (Duda et al., 2011).

FUNCTIONS OF CRLs IN DNA REPLICATION AND CELL CYCLE PROGRESSION

CRLs serve key functions in the regulation of chromosome duplication, modulating crucial steps in the assembly and disassembly of the DNA replication machinery during normal growth and in response to perturbed replication. Roles for cullin-based ring E3 ligases in DNA replication and cell cycle progression have been recently extensively discussed elsewhere (Abbas and Dutta, 2017), and the involvement of CRLs in the early stages of DNA replication in various organisms are briefly summarized below.

The first step in the DNA replication process in all eukaryotes is the loading of the origin recognition complex (ORC) and recruitment of the MCM2-7 helicase complex by the licensing factors CDC6 and CDT1. This complex assembly occurs during late mitosis and the early G1 phase, to form an inactive pre-replication complex (pre-RC). Pre-RCs are subsequently activated by the recruitment of additional factors and by cyclin-dependent kinases (CDKs) and DBF4-dependent kinases (DDKs) (Parker et al., 2017). CRL-controlled levels and/or activities of

proteins involved in pre-RCs assembly and activation are crucial for the orderly initiation of DNA replication and the prevention of re-replication.

In yeast, RTT101, the human CUL4 homolog, modulates MRC1 (human claspin homolog) interaction with the CMG (Cdc45-MCM-GINS) helicase (Buser et al., 2016). RTT101 deletion leads to reduced association of both the replicative helicase MCM and FACT, a complex that assemble or partially disassemble nucleosomes, to replication origins (Han et al., 2010). Cells lacking RTT101 are defective in DNA replication through DNA damaged sites (Zaidi et al., 2008). The yeast CDC6, crucial for pre-RC licensing is degraded in a CRL-dependent pathway (Drury et al., 1997). CRL-induced CDC6 degradation is required to prevent DNA rereplication (Ikui et al., 2012). The yeast CMG is ubiquitinated and disassembled by DIA2 (a F-box protein related to the human CUL1/F-box complex) that binds replication origins (Koepp et al., 2006; Maculins et al., 2015).

In vertebrates, CRLs demonstrate similar functions. In *Xenopus*, CUL2 is a key player during the termination of DNA replication, disassembling the CMG helicase complex (Sonneville et al., 2017). In mammalian cells, MCM3, an essential subunit of the replicative DNA helicase, is a CRL3 substrate (Mulvaney et al., 2016). Both CRL1 and CRL4 can play important roles in the regulation of pre-RC assembly by modulating the chromatin association of two essential licensing factors, CDC6 and CDT1. CDC6 is targeted for degradation by the CRL4-CDT2 and the CRL1-CyclinF complex in S phase (Clijsters and Wolthuis, 2014) and G2-M (Walter et al., 2016) respectively. Targeted degradation of CDT1 in S-phase is shared between CRL1/SKP2 (at the G1/S transition) and CRL4/CDT2 (during S-phase) (Kim and Kipreos, 2007; Pozo and Cook, 2016; Abbas and Dutta, 2017). Thus, deregulation of CRLs in the nucleus leads to CDT1 accumulation and, in turn, to DNA re-replication and genomic instability (Kim and Kipreos, 2007; Pozo and Cook, 2016). CRL4-CDT2 also mediates the degradation of the histone H4 methyltransferase SET8, an enzyme catalyzing the monomethylation on lysine 20 of histone H4 that allows the loading of the pre-RC component ORC1 and the ORC-associated protein ORCA (Beck et al., 2012). SET8 degradation is essential to prevent DNA re-replication (Abbas et al., 2010). Another protein involved in DNA replication initiation include MMSET, a histone methyltransferase degraded during S phase in a CRL4 dependent manner and necessary for the optimum association of pre-replication factors (Evans et al., 2016). The CRL4-interacting DCAF, RepID (DCAF14/PHIP) binds a subset of replication origins and is essential for initiation from those origins (Zhang Y. et al., 2016). The mechanism by which a DCAF can facilitate initiation is unclear, however recent evidence suggests that the CRL4/CUL4B complex facilitates replication licensing through a CUL4B-CDK2-CDC6 cascade, leading to the upregulation of CDK2 and protecting CDC6 from degradation (Zou Y. et al., 2013).

CRLs also control DNA replication via indirect mechanisms. Increased CDK1/2 activities, necessary for origin firing, occur in late G1 and at the G1/S transition through the CRLs-controlled degradation of CDK inhibitors such as p27, p21, and p57 (reviewed in Abbas and Dutta, 2017). Following DNA replication

TABLE 1 | Non-exhaustive list of CRLs substrates.

CRLs	Substrates	Receptors	Substrate roles	References
CRL1	EMI1/Cyclin A	β-TrCP1	Regulates mitosis entry	Guardavaccaro et al., 2003
CRL1	CDC25A	β-TrCP1/2	Required for progression from G1 to the S phase of the cell cycle	Busino et al., 2003
CRL1	CDC25B	β-TrCP1/2	Required for entry into mitosis	Kanemori et al., 2005; Uchida et al., 2011
CRL1	WEE1	β-TrCP1/2	Cell cycle progression, G2/M transition	Watanabe et al., 2004
CRL1	Cyclin D1	β-TrCP1/2	Progression through the G1 phase of the cell cycle	Wei et al., 2008
CRL1	Claspin	β-TrCP1/2	Checkpoint mediated cell cycle arrest in response to replication stress and DNA damage	Peschiaroli et al., 2006
CRL1	PR-SET7/SET8	β-TrCP1/2	Epigenetic regulation/Histone modification	Wang et al., 2015
CRL1	Securin	β-TrCP	Prevent sister chromatin separation	Limón-Mortés et al., 2008
CRL1	SAK/PLK4	β-TrCP1	Prevents centrosome amplification	Cunha-Ferreira et al., 2009
CRL1	MCL1	FBXW7	Involved in apoptosis regulation	Wertz et al., 2011
CRL1	P27^{KIP1}	FBXL1/SKP2	Involved in cell cycle progression	Nakayama et al., 2001
CRL1	P21^{Cip1}	FBXL1/SKP2	Cell cycle progression	Bornstein et al., 2003
CRL1	P57^{Kip2}	FBXL1/SKP2	Inhibitor of several G1 cyclins	Pateras et al., 2006
CRL1	P130	FBXL1/SKP2	Heterochromatin formation	Bhattacharya et al., 2003
CRL1	CDT1	FBXL1/SKP2	DNA replication licensing factor	Li et al., 2003
CRL1	Cyclin D	FBX4/FBXL1/SKP2	G1/S transition	Yu et al., 1998; Gong et al., 2014
CRL1	Cyclin G2	FBXL1/SKP2	Regulation of cell cycle progression	Xu et al., 2008
CRL1	Cyclin D2	FBXL2	Progression through the G1 phase of the cell cycle	Chen et al., 2012b
CRL1	Cyclin D3	FBXL2	G1/S transition	Chen et al., 2011
CRL1	Cyclin E	FBXW7	G1/S transition	Gong et al., 2014
CRL1	P85beta	FBXL2	Control PI3K signaling cascade	Kuchay et al., 2013
CRL1	VPS34	FBXL20	Catalytic subunit of the PI3K complex kinase	Xiao et al., 2015
CRL1	JMJD2A	FBXL4	Epigenetic regulation/Histone modification	Das et al., 2014
CRL1	CITED2	FBXL5	Transcription regulation	Machado-Oliveira et al., 2015
CRL1	Aurora A	FBXL7	Regulates mitosis	Coon et al., 2012
CRL1	Aurora B	FBXL2	Regulates mitosis	Chen B. B. et al., 2013
CRL1	CaMK1	FBXL12	Calcium/calmodulin-dependent protein kinase	Mallampalli et al., 2013
CRL1	CDC6	FBXO1/Cyclin F	DNA replication licensing factor	Walter et al., 2016
CRL1	DCAF2/CDT2	FBXO11	Efficient progression through S and G2/M phases	Abbas et al., 2013
CRL1	UHRF1	β-TrCP/FBW1A	Maintenance of DNA methylation patterns during DNA replication	Chen H. et al., 2013
CRL2	HIF1alpha	VHL	Response to hypoxia	Ohh et al., 2000
CRL2	SPRY2	VHL	May function as an antagonist to several growth factors	Anderson et al., 2011
CRL2	RNA polII subunit	VHL	Transcription	Kuznetsova et al., 2003
CRL2	CKI1	LRR1	Casein kinase involved in several cellular functions	Merlet et al., 2010
CRL2	P21^{Cip1}	LRR1	Cell cycle progression	Starostina et al., 2010
CRL2	TRA1	FEM1	Epigenetic regulation/Histone modification	Shi et al., 2011
CRL2	TOPBP1	–	DNA replication	Blackford et al., 2010
CRL2	H2B	–	Core component of the nucleosome	Li et al., 2010
CRL3	NRF2	KEAP1	Negative regulation of antioxidant response	McMahon et al., 2003
CRL3	WNK4	KEAP1	Blood pressure regulation	Andérica-Romero et al., 2014
CRL3	DAXX	SPOP	Transcription repressor	Sakaue et al., 2017
CRL3	MCM3	KEAP1	DNA replication	Mulvaney et al., 2016
CRL3	PP2A	–	Resistance of cancer cells to death receptor-induced apoptosis	Xu et al., 2014
CRL3/CRL4	TOP1	–	DNA replication, transcription	Zhang et al., 2004; Kerzendorfer et al., 2010
CRL4	CDT1	DCAF2/CDT2	DNA replication licensing factor	Zhong et al., 2003; Higa et al., 2006
CRL4	P21^{Cip1}	DCAF2/CDT2	Cell cycle progression	Abbas et al., 2008; Nishitani et al., 2008
CRL4	PR-SET7/SET8	DCAF2/CDT2	Epigenetic regulation	Jørgensen et al., 2011

(Continued)

TABLE 1 | Continued

CRLs	Substrates	Receptors	Substrate roles	References
CRL4	P27^{Xic1}	DCAF2/CDT2	Cell cycle arrest	Chuang and Yew, 2001
CRL4	CKI1	DCAF2/CDT2	Casein kinase involved in several cellular functions	Kim et al., 2008
CRL4	E2F	DCAF2/CDT2	Cell cycle regulation	Shibutani et al., 2008
CRL4	TDG	DCAF2/CDT2	DNA glycosylase	Slenn et al., 2014
CRL4	CHK1	DCAF2/CDT2	Checkpoint mediated cell cycle arrest in response to DNA damage	Huh and Piwnica-Worms, 2013
CRL4	Histone H2A, H3, H4, DDB2	DDB2	Core components of the nucleosome	Kapetanaki et al., 2006
CRL4	SLBP	DCAF11	Histone biosynthesis regulation	Djakbarova et al., 2016
CRL4	CK1alpha	CRBN	Casein kinase involved in several cellular functions	Krönke et al., 2015; Petzold et al., 2016
CRL4	ZFP91	CRBN	E3 ubiquitin protein ligase	An et al., 2017b
CRL4	APP	CRBN	Cell surface receptor	Del Prete et al., 2016
CRL4	IKZF1, 3	CRBN	Transcription	Krönke et al., 2014
CRL4	Merlin	DCAF1/VprBP	Probable regulator of the Salvador/Warts/Hippo (SWH) signaling pathway	Huang and Chen, 2008
CRL4	FOXM1	DCAF1/VprBP	Transcription	Wang et al., 2017
CRL4	MCM10	DCAF1/VprBP	Replication initiation factor	Kaur et al., 2012
CRL4	TSC2	FBXW5	Regulator of several GTPases	Hu et al., 2008
CRL4	MMSET	DCAF2/CDT2	Epigenetic regulation	Evans et al., 2016
CRL4	LIG I	DCAF7	DNA replication	Peng et al., 2016
CRL4	p12 subunit of DNA polymerase δ	DCAF2/CDT2	DNA replication	Zhang et al., 2013
CRL4/CRL1	CHK1	?	Checkpoint mediated cell cycle arrest in response to DNA damage	Lampert et al., 2017; Tu et al., 2017
CRL4	SLBP	WDR23/DCAF11	Stem-loop binding protein	Lampert et al., 2017
CRL4	FBH1	DCAF2/CDT2	Helicase with a role in response to stalled/damaged replication fork	Bacquin et al., 2013
CRL4	ORCA/LRWD1	?	G1/S transition. Recruits and stabilizes replication origin complexes	Shen and Prasanth, 2012
CRL4	PCNA	?	DNA replication	Lo et al., 2012
CRL4	p53	DCAF2/CDT2	Transcription/apoptosis	Banks et al., 2006
CRL5	iNOS	SOCS	Nitric oxide production	Kuang et al., 2010; Nishiya et al., 2011
CRL5	TRII	SOCS	Enhanced migration and invasion of tumor cells by SOCS silencing	Liu et al., 2015
CRL5	GHR	SOCS	Regulation of growth hormone signaling	Bullock et al., 2006
CRL5	TRAF6	SOCS	Regulation of lipopolysaccharide signaling	Zhu et al., 2016
CRL7	Cyclin D1	FBXW8	Cell cycle arrest	Okabe et al., 2006
CRL7	IRS1	FBXW8	Regulation of insulin signaling	Xu et al., 2008
CRL7	GRASP65	FBXW8	Maintenance of the Golgi apparatus integrity	Litterman et al., 2011
CRL7	EAG1	FBXW8	Potassium channel modulation	Hsu et al., 2017
CRL9	Cytochrome C	?	Promotes cell survival	Gama et al., 2014
CRL9	Survivin	?	Genome integrity maintenance	Li et al., 2014

initiation, cell cycle progression is also controlled, in part, by the CRL1-timely degradations of the CDK positive regulators cyclin E (for S phase progression), cyclin A, cyclin D1, and WEE1 (for G2 progression) (Watanabe et al., 2004; Abbas and Dutta, 2017). Similarly, progression over mitosis is ensured through EMI degradation by the CRL1- β-TrCP1 complex, leading to increased activity of the anaphase promoting complex/cyclosome (APC/C), an E3 ubiquitin ligase utilizing the cullin-like scaffold protein APC2. Other CRL-targeted proteins associated with cell cycle progression include Claspin, PCNA, MCM10, the DNA polymerase alpha, and histones H2A, H2B, H3, H4 among others (**Table 1**).

CULLIN-BASED RING E3 LIGASES AND CANCER

Since CRLs play critical roles in a myriad of biological processes, it is reasonable to think that the deregulation of cullins and/or other CRLs components can play a major role in cancer

progression. While cullin deregulation (mostly upregulation) have been observed in cancer, downregulation or suppression of some of the CRL components can lead to tumor suppression. Deregulated expression of cullins and cullin-associated factors may occur through CpGs methylation, gene coding region mutations, or promoter deletion and/or micro-RNA-induced silencing among others. Below are several examples linking deregulation of CRLs to cancer.

CUL1-Based Ubiquitin Ligase Complexes

CRL1 is the most studied cullin-associated complex in the context of cancer (**Figure 2**). Deregulated CUL1 expression was reported in lung and gastric cancers (Le Gallo et al., 2012) and during the early stages of melanoma development (Lee et al., 2010). A myriad of CRL1-associated F-box proteins and their substrates are involved in cancer (for a review, see Kitagawa and Kitagawa, 2016).

Altered expression and mutations in several F-box proteins, including SKP2, are hallmarks of several cancers. SKP2 (FBXL11) is part of the FBXL subfamily that comprises 22 members (FBXL1 to FBXL22) containing an F-box motif and a C-terminal Leu-rich repeat (LRR) domain. SKP2, which has been characterized as an oncoprotein, is by far the most studied F-box protein of the FBXL subfamily. SKP2 associates with the SCF (SKP1-Cullin1-F-box) complex and targets p27 for degradation, with major developmental consequences in mice (Nakayama et al., 2004). The absence of SKP2 in mice results in the accumulation of p27, nuclear enlargement, cell polyploidies, and centrosome overduplication (Nakayama et al., 2000), phenotypes that disappeared in $SKP2^{-/-}/p27^{-/-}$ double-mutant mice (Nakayama et al., 2004). Chemical-induced skin tumorigenesis is inhibited in SKP2(−/−) mice (Sistrunk et al., 2013) whereas overexpression of *Skp2* in mice led to tumor development in the prostate (Shim et al., 2003), suggesting that a SKP2 deregulation-induced oncogenesis may be tissue specific. SKP2 is also a crucial mediator of BCR-ABL-induced

leukemogenesis (Agarwal et al., 2008). SKP2 is deregulated and correlated with poor prognosis in a wide array of human cancers including breast, prostate, colon, lung, brain, gastric, and blood (Frescas and Pagano, 2008; Zheng et al., 2016). Therefore, the inhibition of SKP2 could be a novel strategy for the treatment of some human cancers.

Other FBXL proteins, also mediate the degradation of substrates involved in cell-cycle progression (**Table 1**). Ectopic expression of FBXL2 in transformed lung epithelia facilitates polyubiquitination and degradation of cyclin D3, leading to G2/M-phase arrest, increased frequency of apoptotic cells, and chromosomal anomalies (Chen et al., 2011). FBXL2 recognizes a canonical calmodulin-binding motif within cyclin D3 and compete with calmodulin for cyclin D3 binding (Chen et al., 2012a). It is thought that FBXL2 targets cyclin D2 for degradation to inhibit cancer cell proliferation. Several patient samples show suppressed expression of FBXL2 together with robust cyclin D2 levels in acute myelogenous leukemia and acute lymphoblastic leukemia (Chen et al., 2012b). Ectopically expressed FBXL2 significantly inhibited the growth and migration of tumorigenic cells and tumor formation in athymic nude mice (Chen et al., 2012a). FBXL2 was also shown to ubiquitinate Aurora B, an integral regulator of cytokinesis that inhibits tumorigenesis (Chen B. B. et al., 2013). FBXL3 was described as a regulator of the circadian rhythm by targeting Cryptochrome (Cry1/Cry2) proteins (Siepka et al., 2007). Since, growing evidence are pointing out that deregulation of the circadian clock plays an important role in carcinogenesis (Savvidis and Koutsilieris, 2012), one putative role for FBXL in cancer could be through the disruptions of normal circadian rhythms.

FBXL proteins are also involved in the epithelial to mesenchymal transition, which often accompanies tumor progression. For example, FBXL5 and FBXL14 inhibit cell invasiveness by targeting SNAIL1 in gastric cancer cells (Vinas-Castells et al., 2010; Wu et al., 2015; Cen et al., 2017). FBXL10 (also known as KDM2B), which contains a JmiC
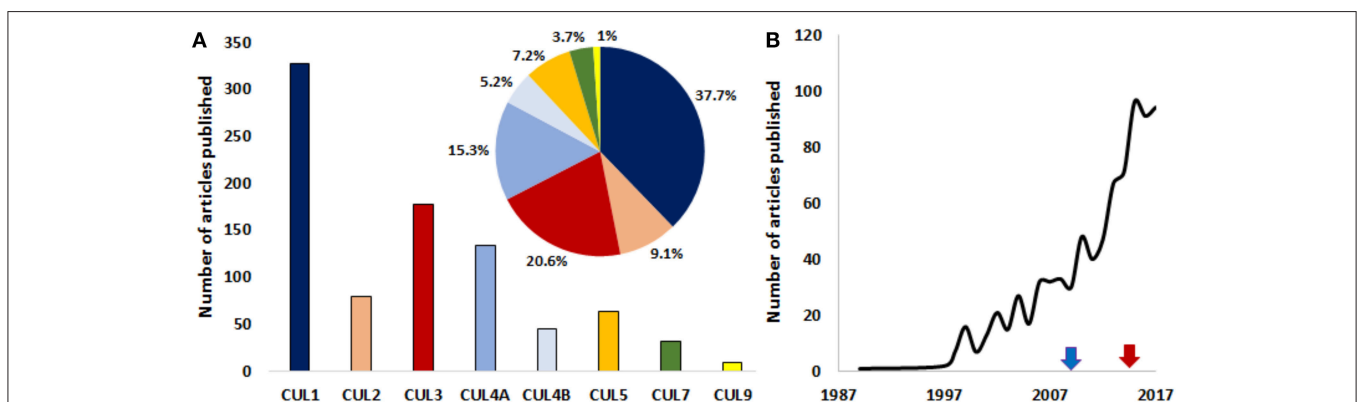
FIGURE 2 | A growing interest for the roles of cullins in cancer. **(A)** Graph depicting the number of published articles studying the role of cullin1, 2, 3, 4A, 4B, 5, 7, and 9 in cancer. The pie chart shows the relative distribution (%) of published articles among the different cullins (the color code is the same as in the bar graph). **(B)** The number of published studies looking at the role of cullins and/or cullin-interacting proteins in the pathology of cancer are increasing exponentially. Blue arrow and red arrows denote the first studies targeting cullins in mice and human respectively. The number of publications for the year 2017 is an estimation made from the number of articles that were published in the first 10 months of the same year. (*Source: PubMed*).

domain (Tsukada et al., 2006) and primarily regulates metabolic and developmental genes (Zheng et al., 2016), is involved in H2AK119 ubiquitination and histone H3K36 demethylation (Wu et al., 2013) and affects TRAIL-induced apoptosis (Ge et al., 2011). The latter observation implies that targeting FBXL10 could overcome resistance to TRAIL treatment in human cancer. Like other CRL1 interacting proteins, FBXL10 role in cancer seems to be tissue specific in humans with higher levels of FBXL10 observed in several types of cancers while it is downregulated in brain tumors (Frescas et al., 2007; Tzatsos et al., 2013). A tissue-specific relationship between FBXL10 and cancer is also observed in transgenic mice. Mice with hematopoietic stem cells overexpressing FBXL10 were shown to develop myeloid or B-lymphoid leukemia (Ueda et al., 2015) whereas FBXL10 depletion was shown to abrogate tumorigenicity in the pancreas (Tzatsos et al., 2013).

Deregulation of the F-box and WD containing protein β-TrCP is associated with several cancers, including breast, colon, pancreatic, liver, gastric, and prostate (Frescas and Pagano, 2008), and both overexpression and mutations in β-TrCPs have been reported in gastric, prostate, breast, pancreas, colon liver, and skin cancer (reviewed in Zheng et al., 2016). In line with these observations, a study using transgenic mice expressing human β-TrCP 1 targeted to epithelial cells under the control of the mouse mammary tumor virus (MMTV) showed that 38% of these mice developed mammary, ovarian, and uterine carcinomas (Kudo et al., 2004). Beyond altering cell cycle progression, β-TrCP1 and β-TrCP2 are involved in the degradation of the transcription factors SNAIL and TWIST and the extracellular matrix fibronectin, all involved in metastasis (Ray et al., 2006; Kitagawa and Kitagawa, 2016). In addition, invasion of human melanoma cells is suppressed by silymarin, a plant flavonoid, in part through β-TrCP-mediated degradation of β-catenin (Vaid et al., 2011). Further evidence linking β-TrCPs to skin cancer is the observation that degradation of IκBα and PDCD4 by β-TrCPs can contribute to the development of skin squamous carcinoma (Dorrello et al., 2006; Gu et al., 2007) while expression of a dominant negative β-TrCP in mouse epidermis confers skin proliferation and apoptosis resistance in response to UVB irradiation (Bhatia et al., 2011). The role for β-TrCPs in carcinogenesis is complex since these proteins also promotes anti-cancer activities by controlling the degradation of several pro-apoptotic proteins such as MCL-1 (Ding et al., 2007), BimEL (Dehan et al., 2009), PDCD4 (Dorrello et al., 2006), pro-caspase 3 (Tan et al., 2006).

Other F-box proteins, including FBXW7, FBXW8, and FBXW9 play roles in carcinogenesis, mainly through regulating the levels of factors involved in cell cycle progression (**Table 1**). FBXW7, a major tumor suppressor, negatively regulates more than a dozen of oncogenic proteins with pivotal roles in cell cycle progression, proliferation, and cell division. FBXW7 also regulates protein degradation involved in DNA damage repair, cell apoptosis and metastasis (for review, see Cheng and Li, 2012) (**Table 1**). The *FBXW7* 4q31.3 locus is deleted in ∼30% of cancers (Knuutila et al., 1999) with a *FBXW7* mutation rate of ∼6% in primary tumors (Akhoondi et al., 2007). FBXW7 mutations and deletions have been described in various type of

tumor types including T-cell leukemia, stomach, pancreas, breast, colon, bladder, prostate cancer, gastric, and cholangiocarcinoma with T-cell leukemia and cholangiocarcima harboring the highest mutations rates of 31 and 35% respectively (reviewed in Cheng and Li, 2012; Zheng et al., 2016). However, mutation in *FBXW7* alone may not be sufficient for carcinogenesis since a recent study showed that both *FBXW7* and *NOTCH1* deregulation may be needed for the induction of human T-ALL (Takeishi and Nakayama, 2014). The *FBXW12* gene coding regions or promoter were found to be deleted in several epithelial ovarian cancers (De la Chesnaye et al., 2015). FBXW8 modulates cancer cell proliferation through cell-type specific cyclin D1 degradation (for review, see Zheng et al., 2016). Thus, FBXW8 is involved in the proliferation of human choriocarcinoma cells via G2/M phase transition with the regulation of CDK1, CDK2, cyclin A, cyclin B1, and p27 expression (Lin et al., 2011). FBXW8 also promotes the degradation of the hematopoietic progenitor kinase 1 (HPK1), a member of mammalian STE20-like serine/threonine kinases that is lost in >95% pancreatic cancer via proteasome-mediated degradation (Wang et al., 2014). The mouse FBXW12 homolog (FBXW15) interacts with histone acetyltransferase binding to the origin recognition complex (HBO1) to mediate its CUL1-regulated ubiquitination (Zou C. B. et al., 2013). Because HBO1 plays a crucial role in DNA replication licensing and cell proliferation, FBXW15 could control DNA replication licensing and cell proliferation.

CUL2-Based Ubiquitin Ligase Complexes

CUL2 is the scaffold protein of the CRL2 complex, recruiting the substrate receptor von Hippel-Lindau protein (pVHL) through the dimer complex EloB and EloC (Pause et al., 1997). pVHL can also be associated to CUL5 (Okumura et al., 2016). A germline mutation in VHL is the basis of familial inheritance of von Hippel-Lindau syndrome, which is characterized by the development of cysts and tumors in multiple organ systems (reviewed in Johnson et al., 2007). Mutations in pVHL or loss of heterogeneity result in high levels of HIF proteins and VHL tumorigenesis (Cassol and Mete, 2015). Deregulation of these two protein is involved in the development of VHL-associated clear-cell renal cell carcinoma (Maynard and Ohh, 2004) with pVHL ectopic expression in VHL$^{-/-}$ renal cell carcinoma leading to suppression of tumor formation in mice (Maynard and Ohh, 2004). HIF-1α is also often overexpressed in several other cancers (Zhong et al., 1999).

In addition to the extensively studied HIF-1α, many other CRL2 substrates have been identified (**Table 1**). HIF-1α triggers a transcriptional response to hypoxia, a key process critical to promote tumor progression and an important determinant of resistance to therapy (Vaupel and Mayer, 2007). Disruption of CRLs components (mutation, gene loss) associates with enrichments of HIF-target genes in several tumor types (Rowbotham et al., 2014). Still, deregulation of CRLs may not solely account for deregulated HIF-1α in cancer since HIF-1α levels are controlled by different signaling mechanisms, including regulation by the HSP90 pathway, the HIF-1 pathway and the MDM2-p53 mediated ubiquitination pathway (Rowbotham et al., 2014; Cassol and Mete, 2015; Masoud and Li, 2015). Since

no CUL2 mutation was found to play a critical role in HIF-1α activation in several cancers (Park et al., 2009), it is likely that HIF-1α loss of homeostasis in cancer is mediated primarily by deregulation of cullin expression rather than cullin point mutations (Zhong et al., 1999). The silencing of another CUL2 substrate, RhoB, is a crucial step driving carcinogenesis (Huang and Prendergast, 2006). In liver cancer, RhoB is targeted for degradation via the CUL2-RBX1 complex, an important effector that drives liver carcinogenesis (Xu et al., 2015). Similarly, CUL2 silencing in HPV16 positive cervical cancer cells resulted in slow growth of xenograft tumors retarding G1-S transition of the cell cycle and favoring apoptosis (Xu et al., 2016). As observed with other cullins, CUL2 deregulation may be driven by microRNAs. For example, CUL2 overexpression in gastric cancer tissues may be driven, in part, by aberrant levels of miR-574-3p (Su et al., 2012), suggesting a role for the CUL2/miR-424 pathway in promoting growth in cancer cells.

CUL3-Based Ubiquitin Ligase Complexes

Alterations of signaling pathways caused by the deregulation of CUL3 and/or CUL3-associated factors can give rise to cancer. For example, CUL3, in complex with the substrate adaptor Ketch-like family member 20 (KLHL20), is thought to promote cancer progression through increased ubiquitination and degradation of the Promyelocytic leukemia (PML) protein (Yuan et al., 2011). Hypoxia may exacerbate PML-KLHL20-driven carcinogenesis, since the promoter of *KLHL20* contains several hypoxia-response elements (Yuan et al., 2011) and PML is a negative regulator of HIF-1 (Bernardi et al., 2006). Thus, degradation of PML by KLHL20 would potentiate a strong induction of several hypoxia pathways. Indeed, KLHL20 expression is elevated in prostate cancer and correlates with HIF-1α upregulation, and PML downregulation (Yuan et al., 2011). It should be noted however that a study with HeLa cells suggests that HIF-2α, not HIF-1α, interacts with KLHL20 and that knockdown of KLHL20 decreased HIF-2α but not HIF-1α protein levels (Higashimura et al., 2011). Although this study did not show directly that KLHL20-mediated protection of HIF-2α from degradation involves CRL3, it suggested that both HIF-1α and HIF-2α may be controlled by CRLs in cancer.

Another Kelch-like family member, KLHL39, is down regulated in cancer and correlates with both low PML expression and cancer progression (Chen et al., 2015). Unlike KLHL20, KLHL39 does not bind CUL3 but acts as an inhibitor by blocking KLHL20-mediated ubiquitination of PML by inhibiting KLHL20 binding to both CUL3 and its putative substrates (Chen et al., 2015). Thus, KLHL39 may act as a tumor suppressor by blocking KLHL20-dependent ubiquitination of PML and other substrates (Yuan et al., 2011).

The CRL3 substrate Kelch-like ECH-associated protein (KEAP1) is a key inhibitor of the transcription factor NRF2 that regulates genes involved in the antioxidant response and drugs detoxification (Chen and Chen, 2016). The CUL3-KEAP1-NRF2 pathway prevents oxidative stress-induced DNA damage and carcinogenesis in normal cells and mediates the response to oxidative stress, cell growth, and survival in cancer cells. The CRL3-KEAP1-NRF2 pathway contributions to cancer

development are reinforced by the observed deregulation of KEAP1 and the presence of CUL3 mutations that could lead to NRF2 overexpression in many cancers (Chen and Chen, 2016 and references therein).

Other dual adaptor/receptor BTB proteins may have a crucial role in maintaining specific metabolic pathways controlled by hormone receptors (Zhuang et al., 2009). Speckle type BTB/POZ protein (SPOP) is one of the highest loci to exhibit loss of heterozygosity in breast cancers (Li et al., 2011) and has high mutation rates in prostate (Kan et al., 2010) and endometrial cancers (Le Gallo et al., 2012). Frequent mutations in SPOP occur in domains that interfere with E3 substrate binding and may affect SPOP's ability to degrade androgen receptors that contribute to cancer development in prostate cancer (An et al., 2014) and progesterone receptors in breast cancer cells (Gao et al., 2015).

CUL4-Based Ubiquitin Ligase Complexes

Deregulation of CUL4A leads to tumorigenesis in transgenic mice (Jia et al., 2017) and the CUL4A locus is often amplified in many human cancers, including hepatocellular carcinomas, pleural mesotheliomas, breast and prostate cancers, squamous cell carcinoma, adrenocortical carcinoma, medulloblastoma, and ovarian invasive carcinoma (Sharma and Nag, 2014 and references therein). CUL4A overexpression in cancer is associated with tumor size, cell proliferation, migration, invasion, and cancer aggressiveness (Song et al., 2015; Deng et al., 2016; Ren et al., 2016; Jia et al., 2017; Nagel et al., 2017). In addition, CUL4A silencing can inhibit cell proliferation and invasion, and induce cell apoptosis. These processes are concomitant with increased expression of p53 and p27 and decreased expression of the metastasis—associated matrix metalloproteinase MMP-2 (Song et al., 2015). CUL4A involvement in tumorigenesis may be directly linked to its pivotal roles in the degradation of tumor suppressors or proto-oncogenic proteins associated with growth regulation, including p21, p73, p150/Sal2 and RASSF1A, N-and c-Myc and c-Jun (Sharma and Nag, 2014; Song et al., 2015). CUL4A may also play a crucial role in the regulation of PAQR3 (progestin and adipoQ receptor family member III), a newly discovered tumor suppressor that exerts its biological function through negative regulation of the oncogenic Raf/MEK/ERK signaling (Qiao et al., 2015).

Overexpression of CUL4B in several cancers such as lung, colon, pancreatic, esophageal, liver, kidney, bladder, and cervical cancer, generally associated with poor patient prognosis, has been reviewed elsewhere recently (Li and Wang, 2017). The critical role for CUL4B in tumorigenesis can be explained by its pleiotropic roles in cellular mechanisms such as cell cycle progression, DNA damage repair and apoptosis (see Li and Wang, 2017 for more details). In cervical carcinoma, CUL4B expression has been shown to be linked to histological grades with high expression related to tumor size, invasion, and metastasis (Yang et al., 2015; Jia et al., 2017; Li and Wang, 2017).

CUL5-Based Ubiquitin Ligase Complexes

Like the CUL3-BTB complex, CUL5 may be involved in hormone receptor homeostasis. CUL5 overexpression led to decreased cell

proliferation of T47D breast cancer cells (Burnatowska-Hledin et al., 2004) and attenuates estrogen receptor alpha and estrogen-dependent growth in a MAPK-dependent manner (Johnson et al., 2007). Similarly, CUL5 is significantly decreased in endometrial cancers with the most aggressive type of cancer displaying the highest CUL5 reduction (Devor et al., 2016). In concordance, CUL5 overexpression led to significantly slower growth in some endometrial cancer cells. CUL5 expression is negatively regulated by miR-19a and miR-19b (Xu et al., 2012), which are highly expressed in cervical cancer cells and are important determinants of the malignant phenotype in those cells, a phenotype that was suppressed when CUL5 3′UTR was deleted. CUL5 is also a direct target of miR-7 in liver cancer through direct miR-7 binding to the CUL5 3′UTR (Ma et al., 2013). CUL5 silencing by miR-7 led to cell cycle arrest and suppression of colony formation, suggesting that the role of CUL5 downregulation in carcinogenesis could be tissue specific.

CUL7-Based Ubiquitin Ligase Complexes

CUL7 was first identified as a novel antiapoptotic oncogene associated with the regulation of p53 levels (Kim et al., 2007). Breast cancers can overexpress CUL7, leading to p53 downregulation (Guo et al., 2014; Men et al., 2014). CUL7 was also identified as a gene involved in liver carcinogenesis through cirrhosis associated with non-alcoholic fatty liver disease, a disease connected with metabolic syndrome. CUL7 maps to the 6p21.1 amplicon characteristic of this type of liver cancer, suggesting that this particular cancer is driven by the anti-apoptotic effect of increased CUL7 through p53 downregulation (Paradis et al., 2013). CUL7 also promotes epithelial-mesenchymal transformation of liver cancer and its high expression in liver tumors is associated with poor prognosis (Zhang D. H. et al., 2016; An et al., 2017a).

CUL9-Based Ubiquitin Ligase Complexes

CUL9 (formerly known as PARC) is a cytoplasmic, p53-binding protein, and a p53-dependent tumor suppressor in mice (Pei et al., 2011) as well as in murine and human leukemic cells (Seipel et al., 2016; Li and Xiong, 2017). CUL9 deletion-induced tumorigenesis tends to be organ specific since mice lacking CUL9 were shown to develop tumors in sarcoma, lung, liver, and ovary only. CUL9's role in protecting genome integrity and tumor suppression is facilitated by mediating the degradation of survivin and cytochrome C in normal and cancer cells (Gama et al., 2014; Li et al., 2014).

TARGETING CRL COMPLEXES IN CANCER THERAPIES

Since cullins are overexpressed in many cancer types, many novel cancer therapy strategies aim to inhibit cullin-ring ligase activity. MLN4924 (pevonedistat), a selective inhibitor of NEDD8-activating enzyme (NAE) structurally related to adenosine 5′ monophosphate that inhibits cullin NEDDylation and CRLs activity, was first shown to inhibit the growth of human colon tumor xenografts in nude mice (Soucy et al., 2009). In promising experiments, the drug was able to induce rereplication

and permanent growth arrest in melanoma cells but not in immortalized non-transformed melanocytes (Benamar et al., 2016). Another NEDDylation inhibitor, TAS4464, is also tested in clinical trials (**Table 2**). NEDDylation inhibitors inactivate CRL E3 ubiquitin ligases and causes the cellular buildup of many substrates involves in different cellular functions (see Oladghaffari et al., 2016 for a review). Most clinical studies involving pevonedistat/MLN4924 or TAS4464 (**Table 2**) are still restricted to phase I and II trials.

As shown in **Table 2**, the first phase I study involving a NEDDylation inhibitor (pevonedistat) investigated both pharmacokinetics and pharmacodynamics in patients with acute myeloid leukemia and myelodysplastic syndromes and demonstrated a modest clinical activity (Swords et al., 2015). Subsequent phase I studies evaluated the use of pevonedistat against relapsed/refractory multiple myeloma or lymphoma (Shah et al., 2016), advanced nonhematologic malignancies (Sarantopoulos et al., 2016), and metastatic melanoma (Bhatia et al., 2016). At the time of writing, there are 11 ongoing clinical trials using MLN4294/pevonedistat targeting both solid tumors (4) and blood cancers (7) (**Table 1** and clinicaltrials.gov). Since MLN4924 sensitizes cancer cells to several chemotherapeutic drugs (reviewed in Oladghaffari et al., 2016), the majority of ongoing trials (10/11) are evaluating pevonedistat in combination with other anti-tumor drugs such as DNA damaging agents such as carboplatin, nucleoside analogs (azacitidine, gemcitabine, decitabine), and tubulin-binding drugs (paclitaxel, vincristine). In many studies, NAE inhibition by pevonedistat was confirmed *in vivo* by the accumulation of cullin-ring ligases substrates, including CDT1 and NRF2 in solid tumors and upregulation of NRF2 gene in blood. In the metastatic melanoma study (Bhatia et al., 2016), an additional panel of NAE-regulated substrates (ATF3, GCLM, GSR, MAG1, NQO1, SLC7A11, SRXN1, TXNRD1) was used to confirm inhibition of NAE in blood and increases in pevonedistat–NEDD8 adducts. CDT1 and NRF2 protein levels were measured in tumor biopsies. In the study related to advanced nonhematologic malignancies, stable disease was observed in 80% of the patients receiving both dexamethasone and pevonedistat, and in 69% of patients receiving pevonedistat alone (Sarantopoulos et al., 2016). In patients with metastatic melanoma (Bhatia et al., 2016), one patient (3%) achieved partial response while 15 patients (48%) showed stable disease.

Several studies have shown the proof of concept by using MLN4924 for increased cancer cell killing by radiation. MLN4924 sensitized head and neck squamous carcinoma cells to ionizing radiation and enhances radiation-induced suppression of xenografts in mice (Vanderdys et al., 2017). MLN4924 also enhanced the susceptibility of nasopharyngeal carcinoma, colorectal, lung, pancreatic, and breast cancer cells to radiation (Oladghaffari et al., 2016, 2017; Wan et al., 2016; Xie et al., 2017). Importantly, MLN4924 was shown to sensitize several types of cancer cells to ionizing radiation with a minimal effect on non-cancerous cells (Wei et al., 2012). Mechanistically, MLN4924-increased radiosensitization may be due to induced G2 cell arrest, apoptosis, delayed DNA repair, and loss of

TABLE 2 | A non-exhaustive list of clinical studies targeting cullin-RING ubiquitin E3 ligases.

Condition	Drug(s)	Measurements	Phase	References or ClinicalTrials.gov identifier
Advanced solid tumors, neoplasms	(14C)-Pevonedistat	1. cumulative excretion of radioactive Pevonedistat in urine and feces/Circulatory and excretory pevonedistat metabolites 2. Report of TEAEs and SAEs	I	NCT03057366
Recurrent AML, therapy-induced AML, untreated or recurrent AML	Pevonedistat plus Decitabine	1. Safety and tolerability of Pevonedistat added to Decitabine 2. MTD of pevonedistat in combination to Decitabine 3. mIR-155 expression, promoter methylation, and mIR-155 target gene expression (SHIP1/PU.1) 4. NF-kappaB expression and enrichment on mIR-155 promoter	I	NCT03009240
Metastatic melanoma	Pevonedistat	1. MTD of 209 mg/m^2 2. Clinical activity: 3% PR, 48% *SD* 3. Pevonedistat plasma concentration increased approximately proportionally with dose from 50 to 278 mg/m^2 after Day 1 intravenous infusion	I	NCT01011530 (*) (Bhatia et al., 2016)
Solid tumors	1. MLN4924 plus Docetaxel 2. MLN4924 plus Docetaxel plus Carboplatin 3. MLN4924 plus Gemcitabine	1. Number of adverse events 2. Time course MLN4924 plasma concentration	I	NCT01862328
Advanced solid tumors	MLN4924 (schedules A and C) MLN4924 + Dexamethasone (Schedule B)	1. MTD of 50 mg/m^2 (schedule A) 50 and 67 mg/m^2 (schedule B and C, respectively) 2. 11/13 patients with > 20% increase in CDT1 and NRF2 CRLs substrates 3. 13/14 patients show NEDD8 adducts in tumor biopsies 4. Clinical activity: 74% SD for schedules B and C	I	NCT00677170 (*) (Sarantopoulos et al., 2016)
AML	MLN4924 plus Azacitidine	1. Safety and tolerability of MLN4924 in combination with Azacitidine 2. Disease response rate 3. 30-day and 60-day mortality rate	I	NCT01814826
Advanced solid tumors	MLN4924 Fluconazole Itraconazole Docetaxel Carboplatin Paclitaxel	1. TEAEs and disease response 2. MLN4924 plasma concentration, blood to plasma ratio. MLN4924 clearance 3. Clinical response	I	NCT02122770
AML, ALL, MDS	MLN4924 Intravenous infusion on days 1, 3, and 5 (schedule A) and 1, 4, 8, and 11 (schedule B)	1. MTD of 59 (Schedule A) and 83 mg/m^2 (Schedule B) 2. Clinical activity: 17% CR/PR (schedule A); 10% CR/PR (schedule B) 3. 32/35 patients with NEDD8 adduct in tumor biopsies 4. Pevonedistat increased within 4–8 h after infusion and returned to baseline within 24 h	I	NCT00911066 (*) (Swords et al., 2015)
Leukemia, MDS, Myeloid, Acute	1. Pevonedistat 2. Pevonedistat plus Azacitidine	1. TEAEs and dose limiting toxicities 2. Overall and complete responses 3. Pevonedistat plasma concentration and clearance	I	NCT02782468

(Continued)

TABLE 2 | Continued

Condition	Drug(s)	Measurements	Phase	References or ClinicalTrials.gov identifier
Relapsed/refractory multiple Myeloma or lymphoma	MLN4924 Intravenous infusion on Week 1, 2, 8, and 9 (schedule A) and 1, 4, 8, and 11 (schedule B)	1. MTD of 110 mg/m^2 (schedule A) and 196 mg/m^2 (schedule B) 2. 11/13 patients with NEDD8 adducts in bone marrow aspirates 3. CDT1 and NRF2 skin and NRF2 mRNA in blood increased in treated patients 4. Clinical activity: 1 patient with PR and 71% SD	I	NCT00722488 (*) (Shah et al., 2016)
Multiple myeloma Non-Hodgkin lymphoma	TAS4464	1. Investigate the safety and tolerability of TAS4464; identify TAS4464 MTD 2. Efficacy of TAS4464, defined as Objective Response Rate (ORR) per IWG criteria (NHL) and IMWG criteria (MM).	I II	NCT02978235
MDS leukemia, CML	1. Azacitidine 2. Azacitidine plus Pevonedistat	1. EVF 2. OS	II	NCT02610777
Non-small cell lung cancer	Pevonedistat plus Docetaxel	1. Response to treatment 2. Median progression free survival time, OS time, and patients who achieve stable disease 3. Toxicities by system organ class	II	NCT03228186
MDS leukemia, CML, AML	1. Azacytidine 2. Azacytidine plus Pevonedistat	1. EVF, OS, partial remission 2. overall response. 6 months and 1 year survival rate	II	NCT02610777
MDS leukemia, CML	1. Azacitidine 2. Azacitidine plus Pevonedistat	1. Overall response and EVF 3. OS 3. Pevonedistat plasma concentration 4. EVF and OS in participants with TP53 mutations or any adverse cytogenetic risk group	III	NCT03268954

EVF, Event-Free Survival; OS, Overall Survival; AML, Acute Myeloid Leukemia; CML, Chronic Myelomonocytic Leukemia. TEAEs, Treatment Emergent Adverse Events; SAEs, Serious Adverse Events; MDS, Myelodysplastic Syndrome; MTD, maximum tolerated dose. CR, complete response; PR, partial response; SD, Stable diseases. (*) Study completed.

radical oxygen species homeostasis (Oladghaffari et al., 2016; Wang et al., 2016). For all these reasons, future clinical trials may expand the use of NAE inhibitors to radiotherapy to treat cancer.

FUTURE DIRECTIONS

Developing a better understanding of the contributions of each Cullin-Ring Ligase complex in cellular homeostasis remains a challenging task. A large portion of the 100,000 different proteins that are present per cell needs to be recycled or eliminated in a timely manner during development or cell cycle progression. The complexity of such a task explains the extreme intricacies of protein degradation, where substrate recognition (or protein modification recognition) is crucial. It also suggests that most of the CRL substrates are yet to be discovered. Future studies are expected to reveal new CRL-interacting factors and new regulatory pathways,

and provide further insights into the existence of regulatory crosstalk among the different CRLs. New roles for cullins in carcinogenesis will assuredly emerge in the near future since the relationship between cancer and some cullins (i.e., CUL7 and CUL9) is still a relatively new concept (**Figure 2**).

While drugs that inhibit all CRLs are currently being validated, it is plausible that future drug development will also target individual CRLs or specific CRLs-interacting factors. For example, a specific SKP2 inhibitor that selectively suppresses the CRL1 E3 ligase activity was reported to exhibit anticancer activity against human tumor xenografts in mice (Chan et al., 2013). In another approach, homo-bivalent molecules aiming to target CRL2-VHL induced preferential dimerization and isoform-selective degradation of VHL (Maniaci et al., 2017). Further development of agents that modulate specific interactions with substrate receptors is expected in the future. Of particular interest are CRLs that play regulatory roles in molecular pathways altered in cancer cells, such as components

of chromatin-associated CRLs that modulate DNA replication. Such a development will benefit from further understanding of the interactions that fine-tune DNA replication, such as selective interactions of groups of replication origins with distinct regulatory proteins, including DCAF members of CRL4 (Zhang Y. et al., 2016; Aladjem and Redon, 2017). Future advances will be likely target CRL-mediated pathways that maintain genomic stability by preventing DNA rereplication and modulate the S-phase DNA damage response via protein degradation.

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